Soil biota in boreal urban greenspace: responses to plant type and age

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Abstract

Plant functional type influences the abundance and distribution of soil biota. With time, as root systems develop, such effects become more apparent. The relationship of plant type and time with the structure and abundance of soil microbial and invertebrate communities has been widely investigated in a variety of systems. However, much less is known about long-term soil community dynamics within the context of urban environments. In this study, we investigated how soil microbes, nematodes and earthworms respond to different plant functional types (lawns only and lawns with deciduous or evergreen trees) and park age in 41 urban parks in southern Finland. As non-urban controls we included deciduous and evergreen trees in 5 forest sites. We expected that microbial biomass and the relative abundance of fungi over bacteria would increase with time. We also expected major differences in soil microbial and nematode communities depending on vegetation: we hypothesized that i) the presence of trees, and evergreens in particular, would support a greater abundance of fungi and fungal-feeding nematodes over bacteria and bacterial-feeding nematodes and ii) the fungi to bacteria ratio would be lowest in lawns, with deciduous trees showing intermediate values. In contrast to our predictions, we showed that old deciduous trees, rather than evergreens, supported the highest fungal abundances and fungal-feeding nematodes in the soil. Consistent with our predictions, microbial biomass in urban park soils tended to increase with time, whereas – in contrast to our hypotheses – fungal-feeding nematode abundance declined. Even in the oldest parks included in the current study, microbial biomass estimates never approximated those in the minimally managed natural forests, where biomass estimates were three times higher. Anecic earthworm abundance also increased with time in urban parks, whereas abundances of fungal-feeding, plant-feeding and omnivorous nematodes, as well as those of epigeic and endogeic earthworms remained constant with time and without any distinct differences between urban parks and the control forests. Our findings highlight that although urban park soils harbor diverse soil communities and

considerable microbial biomass, they are distinct from adjacent natural sites in community composition and biomass.

Highlights

- In cities, soil communities under different plant types diverge increasingly with time
- Microbial biomass associated with deciduous trees increases with park age
- Earthworm biomass increases with park age
- Adjacent non-urban sites maintain a greater microbial biomass than urban parks

1. Introduction

Urbanized areas are rapidly expanding at the expense of natural habitats. Urban green spaces, including public parks, assume a pivotal role as surrogates for these lost natural environments. These green spaces provide a vast array of ecosystem services (Costanza et al., 1997; Bolund and Hunhammar, 1999) including carbon and nitrogen sequestration (Raciti et al., 2011; Setälä et al., 2016), storm water interception and purification (Valtanen et al., 2015), biodiversity and climate regulation (Bolund and Hunhammar, 1999). These ecosystem services depend strictly on the soil, and in turn on the soil biota hosted therein.

Soil microbes and invertebrates are directly linked to biogeochemical processes that take place in the soil and thus promote a variety of soil-derived ecosystem services (Lavelle et al. 2006; Balser and Firestone, 2005; Blouin et al., 2013 Ledin, 2000; Haritash and Kaushik, 2009). Yet, the abundance and distribution of soil biota are often directly linked with the distribution of plant species, and indirectly to soil properties that are modified by the plants (Wardle et al., 2004) over time (Bardgett et al., 2005). Although the successional trajectories of soil communities in relation to plant type and soil characteristics over time have been widely investigated in, e.g., primary (Ohtonen et al., 1999; Doblas-Miranda et al., 2008; Brown and Jumpponen 2014) and secondary succession (Pižl, 1992; Maharning et al., 2009), much less is known about these dynamics in urban park soils.

The linkage between plant functional types and soil microbial and invertebrate communities has received substantial interest in contemporary soil- and ecosystem ecology (Orwin et al., 2010; Thomson et al., 2010). There is also a growing body of literature describing soil biota in urban parks (microbes: Baxter et al., 1999; Xu et al., 2014; Ramirez et al., 2014; Hui et al. 2017a, b; nematodes: Pavao-Zuckerman and Coleman 2007; Amossé et al., 2016; earthworms: Steinberg et al., 1997; Smetak et al., 2007; Amossé et al., 2016). In a nutshell, these studies collectively provide evidence that urbanization can substantially change in soil microbial and faunal communities. Yet, there is a paucity of studies that simultaneously account for

different trophic groups in large and well-replicated experimental designs within the context of urban ecosystems.

Plant species identity is linked to the quantity and quality of inputs provided to the soil, either via litter deposition or through root exudates, which in turn are largely responsible for the composition of soil microbial (Grayston et al., 1998; Bardgett and McAlister, 1999; Marschner et al., 2004), nematode (Ilieva-Makulec et al., 2006) and earthworm communities (Curry, 2004). Whilst plants that produce labile, nitrogen-rich litter, such as grasses, herbs and deciduous trees often support bacterial-dominated soil microflora, plants producing more recalcitrant litter, such as evergreens, more commonly support fungal-based soil food webs (Wardle et al. 2004). Evergreen trees are adapted to low nutrient availability and thus have leaves with low nutrient contents (Kattge et al. 2011). In contrast, deciduous broadleaf trees have higher foliar nutrient content (Kattge et al. 2011) with *Tilia cordata* and *Acer platanoides* particularly in particular producing high quality litter (Aerts and Chapin 2000, Hobbie et al. 2014). Moreover, soil acidification promoted by evergreen trees (Setälä et al. 2016) is also associated with the prevalence of fungi over bacteria (Bååth and Anderson 2003). Finally, the quantity of root exudates, in boreal systems, tends to be higher in evergreen trees than deciduous trees (Gower et.al 2001), suggesting that evergreen trees may allocate a higher percentage of net primary productivity to, i.e. ectomycorrhizal fungi than do deciduous trees. Such plant-associated distinctions in soil microbial communities are reflected in the relative proportions of bacterial- and fungal-feeding fauna (Trofymow and Coleman, 1982; de Vries et al., 2013). This is particularly interesting as fungal-based food webs are characterized by slow nutrient cycling and a high capacity to retain nutrients, whereas bacterial dominated food webs are characterized by high nutrient turnover and nutrient leaching (de Vries et al., 2006; de Vries et al., 2012). Consequently, the ratio between fungi and bacteria in the soil is crucial when considering fundamental ecological processes such as the rate of organic matter decomposition (Coleman et al., 1983; Wardle, 2002; Moore et al., 2005; Paterson et al., 2008) and thus carbon and nutrient sequestration.

The type and availability of plant-derived resources can also change during plant community succession (Berendse, 1990; Knops and Tilman, 2000). As soil organic matter (OM), C and N accumulate over time, so too do the biomasses of soil microbes (Zak et al., 1990; Ohtonen et al., 1999), nematodes (Háněl, 2010) and earthworms (Pižl, 1992). Changes in microbial abundances are not only quantitative, but also qualitative. For instance, the relative abundance of fungi over bacteria tends to increase with time (Ohtonen et al., 1999; Zeller et al., 2001; Bardgett and Walker, 2004). Furthermore, temporal changes in the fungal to bacterial ratio during succession are often also mirrored by changes in the ratio of fungal feeding to bacterial feeding nematodes (Brzeski, 1995; Ferris and Matute, 2003; Háněl, 2010). Only a few studies have investigated such successional trajectories of soil biota in urban parks (Smetak et al., 2007; Amossé et al., 2016; Hui et al., 2017a, b).

This study is part of a larger project that aims to shed light on the influence of divergent plant types on the physico-chemical and biological soil characteristics in urban parks of diverging ages. Our previous work has shown that plant type and park age are strong determinants of soil characteristics (Setälä et al., 2016; Setälä et al. 2017) and soil microbial community composition (Hui et al., 2017a, b). However, the response of microbial biomass, a measure that strongly relates to the functional activity and capacity of soils, to plant type and park age remains unresolved. Moreover, in this study, we incorporate the microbial consumer responses – key actors in providing soil-based ecosystem services and contributing to nutrient turnover (Wardle et al., 2004). We aim to investigate how park age and plant functional type affect the biomass of soil microbes and two important functional groups of soil fauna: nematodes and earthworms.

Given the clear effects that plant functional type and park age have on soil properties (see Setälä et al. 2016; Setälä et al. 2017) we tested the following hypotheses: plant functional type affects the soil food web so that i) evergreen trees producing recalcitrant litter promote an increase of fungal biomass over bacterial biomass and ii) deciduous trees, the lawn in particular, producing more labile litter, promote the establishment of bacterial biomass. Then we test iii) if changes in the soil microbial community also cascade up to

higher trophic levels. We expect that higher densities of fungal feeding nematodes (compared to bacterial feeders) associate with evergreen trees and higher densities of earthworms associate with lawns and deciduous trees. Also, we test iv) whether time since park construction promotes changes in soil microbial and invertebrate community structure and abundance. We hypothesize that soil biota in old parks resemble natural communities more than in young parks. This is because the capacity of plants (especially trees) to modify soils is park-age dependent (Setälä et al. 2016, 2017) with young parks not having had the time to develop plant-soil interactions that are typical of natural forests.

2. Materials and Methods

2.1. Study area

The study was conducted in two municipalities in the boreal forest zone in southern Finland; Helsinki metropolitan area (60° 10´15´´N 24° 56´ 15´´E, with a population of ca. 1.4 million people) and Lahti (60° 58' 57 N 25° 39' 41 E, population ca. 110 000). Winters are cold and wet, while rainfall is moderate all year round. The annual mean temperature is 5.3°C in Helsinki and 4.5°C in Lahti; the annual mean precipitation is 628 mm in Helsinki and 636 mm in Lahti. Summer lasts for approximately 110-120 days, winter 135-145 days and the temperature can span from -35 to +35°C (Finnish Meteorological Institute). The Helsinki and Lahti regions are classified by NRCS (National Resource Conservation Service) as having soils of primarily the Spodosol suborder. However, all urban parks in the two cities are constructed and none showed detectable signs of podzolisation at the time of sampling.

We selected 41 parks in the two cities and five additional control forests (see Setälä et al., 2016 and Hui et al., 2017a for details). Parks of three ages were selected: young parks (between 7 and 15 years old), intermediate parks (ca. 50 years old) and old parks (> 100 years). Control forests, situated in the outskirts of the city of Lahti, are typified as unmanaged, conifer and linden

dominated forests (> 80 years of age). Park size varied from one to several hectares. The selected parks were subjected to routine maintenance, including mowing (mowing residues not removed) and raking of tree leaves in the fall. However, the parks were not irrigated or commonly fertilized. Until the early 1990s, some of the older parks in the city of Lahti were occasionally fertilized - commonly with saltpeter (N, P, K, S), while some of the park lawns in Helsinki have received and still receive light refurbishment fertilization.

In each park we selected, where possible, three vegetation-types: deciduous (represented by *Tilia* x *vulgaris* 93% and *Acer platanoides* 7%) and evergreen trees (spruce, *Picea sp.* 43.3%, *Abies sp.* 20%, *Pseudostuga menziesii* 13.3%, *Pinus sylvestris* 13.3%, *Larix sp.* 10%) and a non-treed lawn with grass (including herbs such as *Trifolium pratense*, *Plantago major*). Lawn cover extended also under tree canopies. In some cases we selected parks including only deciduous trees and lawn or only evergreen trees and lawn. The control sites never had lawns, but deciduous (forest linden, *Tilia cordata*) and evergreen trees (Norway spruce, *Picea abies*) were always present at each site. In the parks, distance between the two tree types was always greater than the height of the nearest tree. Plant age was considered as coinciding with park age, except for young parks, where ca. 10 year old saplings were planted at the time of park construction. In order to have at least 10 replicates per park age and plant functional type, we selected 41 urban parks, resulting in 91 sampling locations as described by Setälä et al. (2016) plus five control forests with 10 additional sampling locations, totaling 101 sampling locations.

2.2. Soil sampling and measurements for edaphic responses

In urban parks, soils were sampled at the edge of the canopy projection of each tree and in the middle of the lawn area (when present) in October 2014. In the control forests, soils were sampled under the canopy projection of each tree in May 2015. Samples were obtained using a metal corer that was sterilized in ethanol between samples. At each sampling point, 3 subsample soil cores were collected and then pooled. The soil from each sample was homogenized and larger stones, roots and fresh or recognizable plant material removed. Samples

for microbial analysis were stored in resalable plastic bags on ice in the field and frozen at -20 °C in the laboratory. Soil pH was measured in a 1/5 (vol/vol) soil/distilled water suspension. Soil was weighed and analyzed for percent moisture by drying for 48 hr at 105 °C. Total carbon (hereafter C) and nitrogen (N) were obtained by dry combustion at 1350 °C using a LECO CNS2000 Elemental Analyzer (0.07% C and 0.09% N detection limits) and reported as percentage dry mass.

2.3. Soil microbial analysis, PLFA

Before analysis, the frozen samples were thawed at room temperature and sieved through a 2 mm mesh to remove leaf litter, rocks, large particles and roots. Then, part of the soil was freeze-dried and an aliquot of 4 g for urban park soil and 2 g for forest soil was taken for phospholipid fatty acids (PLFA) analysis. Fatty acids (FA) were extracted following a modified procedure as described in Macnaughton et al. (1997), Bligh and Dyer (1959), Frostegård et al. (1991) and White et al. (1979). The extraction was performed using a Dionex ASE 350 machine (Thermo Scientific). Briefly, the samples were mixed with diatomaceous earth and placed in the extraction vessels between two cellulose filters prewashed in chloroform. FA were extracted with Bligh and Dyer (methanol/chloroform/citrate buffer, 2/1/0.8 vol/vol/vol) at 80 °C and the samples underwent two static cycles of 15 min each. Once FA acids were extracted, chloroform and citrate buffer were added, for a final volume of chloroform/methanol/citrate buffer of 1/1/0.9, vol/vol/vol. The extracts were left overnight to separate the aqueous phase from the lipidic phase. The supernatant was discharged and the lower phase collected, which was then evaporated under a flux of N_2 at 40 °C. The resulting pellet was suspended in chloroform and applied to silica columns (Bond Elut LRC, Agilent). Chloroform, acetone and methanol were applied in sequence to the column to separate neutral lipids, glycolipids and phospholipids. The extracts were then dried under N_2. The phospholipid fraction so obtained was methylated and the fatty acid methyl esters extracted with hexane/chloroform (4/1, vol/vol), dried under N_2 and redissolved in 1 ml hexane. We used 19:0 (methyl

nonadecanoate) as an internal standard. All the solvents used were of GC grade and glassware was baked in an oven at 450 °C for 4 h prior to use.

The samples were analyzed in a Shimadzu GCMSQP2010 Ultra with an Agilent J&WDB23 column. The column oven temperature was set to 60 °C and the injection temperature was 250 °C in splitless mode. MS ion source temperature was 200 °C and the interface temperature was 250 °C. As reference library we used the bacterial acid methyl ester (BAME) mix (SigmaAldrich), while the Supelco 37 Component FAME mix (SigmaAldrich) was used for calibration for PLFA biomass calculation. Peaks were cross-checked against the NIST spectral library. A total of 29 PLFAs were identified. Of the identified peaks, the following were considered to be of bacterial origin: i15:0, 15:0, 17:0, 17:0cy (eubacteria); a15:0, i16:0, i17:0 (gram-positive bacteria); 16:1ω9, 18:1ω7 (gram-negative bacteria). The PLFA 18:2ω6 was considered to be mainly of fungal origin (Frostegård and Bååth, 1996). PLFAs were expressed as $\mu g\ g^{-1}$ soil dry weight. The totality of identified peaks was used to investigate coarse microbial community changes, as explained in section 2.5 below. The microbial to fungal (F/B) ratio was calculated as the ratio of PLFA 18:2ω6 to bacterial PLFAs.

2.4. Soil Fauna

2.4.1. Nematodes

Nematodes were collected in early June 2016 in Lahti, and in July-August 2016 in Helsinki. Similarly to the soil sampling, nematodes were collected at the edge of the canopy projection of each tree and in the middle of the lawn (when present) area. In the control forests, soils were sampled under the canopy projection of each tree type. Each sample consisted of six pooled 5 cm diameter soil cores of the uppermost 10 cm. The samples were pooled to make one composite sample and immediately placed in plastic bags and stored in a cooler. In the laboratory, the soil was gently mixed and 10 g (fresh weight) of soil was used for nematode extraction. Nematodes were extracted for 48 h without lights/heating following the wet funnel method described in Sohlenius

(1979). Nematode feeding guilds were assigned following Yeates et al. (1993). We calculated the nematode channel ratio (NCR) as described in Yeates (2003) and the maturity index (MI) as in Bongers (1990). NCR is calculated as the ratio of bacterial feeding (BF) to bacterial-feeding plus fungal-feeding (FF) nematodes: BF/(BF+FF), and is constrained to have values between 1 (totally bacterial-mediated) and 0 (totally fungal-mediated) (Yeates, 2003). MI is the sum of the relative abundance of each taxon multiplied by their colonizer-persister (c.p.) value. The c.p. value of each family was assigned following Bongers (1990, 1999). MI provides information on the degree of environmental disturbance, with higher values indicating a less disturbed environment.

2.4.2. Earthworms

Earthworms were collected in August 2016 close to where soil microbes and nematodes were sampled. We followed a modified version of the hot mustard liquid method described by Gunn (1992). Each sample consisted of two (in public parks) or four (in control sites) 25 × 25 cm frames irrigated with a hot mustard liquid. The hot mustard slurry was prepared by mixing 15 g of hot mustard powder (Colman's powder) in 100 ml of water, and then allowing it to sit for at least 4 h. Immediately prior to sampling, the mustard slurry was added to approximately 3.5 L of tap water in a watering can. This 3.5 L of mustard water was applied to each frame over a span of 15–20 min. The collected worms were immediately placed in tap water for some minutes, then placed into plastic bags containing moist tissue paper, and stored in a cooler. In the laboratory the earthworms were placed in a refrigerator and stored for 48 h to empty their guts. The worms were then identified, weighed and stored in 4% formaldehyde solution.

2.5. Data analysis

Generalized linear mixed models (GLMM) were used to investigate the relationship between our focal response variables (see below) and park age (a

339factor with three levels; young, intermediate, old), plant functional type (a
340factor with three levels; evergreen, deciduous, lawn), soil pH, % soil C, % soil N,
341C/N ratio, soil OM, and % soil moisture. Our response variables were: total
342microbial biomass (PLFA), PLFA fungal/bacterial ratio, PLFA fungal marker
343(18:2ω6), PLFA gram-/gram+ ratio; nematode NCR ratio, BF nematode, FF
344nematode, PF (plant-feeding) nematode, PO (predators and omnivores)
345nematode, anecic earthworm *Lumbricus terrestris* abundance and endogeic and
346epigeic earthworms (*Lumbricus rubellus*, epigeic; *Allolopophora caliginosa*,
347endogeic; *Octolasion cyaneum*, endogeic - collected in one sample only;
348*Allolophora chlorotica,* endogeic - collected in one sample only; *Dendrobaena*
349*octaedra*, epigeic). These GLMMs did not include the control forest samples in
350Lahti. Response variables were log or square-root transformed, when
351necessary, to satisfy assumptions of normality. In the GLMM analysis, city was
352considered a random effect, with park identity nested within city. We
353performed a stepwise model selection procedure by removing insignificant
354predictor variables, one at a time, if their p-values were greater than 0.05 and if
355the AIC subsequently decreased. However, park age and vegetation functional
356type were always retained in the final model, irrespective of significance. Then,
357we compared the control forest with old parks in Lahti, using ANOVA, with
358park age and vegetation functional type (and their interaction) as factors.
359Lawns were excluded from this analysis. Finally we evaluated whether FF
360nematodes, BF nematodes and NCR were correlated, respectively, with PLFA
36118:2ω6, bacterial PLFA and the PLFA fungal/bacterial ratio, using Pearson
362correlation.

363 Microbial (based on the relative abundance of PLFA biomasses) and
364nematode community structures were assessed using nonmetric
365multidimensional scaling analysis (NMDS). The earthworm community was
366dominated by one species, resulting in low species richness and deemed
367unsuitable for NMDS. For the NMDS analysis we used Euclidean distances for
368PLFAs and Bray-Curtis distances for nematodes. The “envfit” function was used
369to assess the significance of the relationship between these communities and
370the environment (factors: city, park age, plant functional type; abiotic
371parameters: pH, % soil C, % soil N, C/N ratio, soil OM, % soil moisture). To test

our specific hypotheses, NMDS analyses were performed on three different datasets; i) we included all park samples to evaluate the effects of plant functional type and park age on microbial and nematode communities; ii) we included only trees belonging to intermediate and old parks to focus on differences mediated by tree type; iii) we included only old deciduous and evergreen trees in Lahti parks and control forests in order to specifically explore differences between urban parks and natural forest. R scripts and data are provided as supplementary material (Supplementary material, S1-S25).

All statistical analyses were performed in R version 3.2.1. (R Core Team, 2015), using the packages vegan (Oksanen et al. 2007), lme4 (Bates et al. 2014), nmle (Pinheiro et al. 2007) and car (Fox et al. 2011).

3. Results

3.1. Microbial biomass and community composition

The GLMM models provided little evidence for systematic responses in PLFA-based total microbial, bacterial and fungal biomasses (18:2ω6) to plant functional type or park age (Table 1, Fig. 1). Rather, these effects highlighted interactions between park age and plant type: PLFA biomasses (total, bacterial, fungal) were lowest under deciduous trees in young parks, yet highest under deciduous trees in old parks (Fig. 1 a, b). Total microbial and bacterial biomasses were mainly negatively correlated with the C/N-ratio (Table 1, Fig. 2a) and positively correlated with % soil C (Fig. 2b). The fungal marker responded positively to % soil N (Table 1, Fig. 2c).

In old Lahti parks, total microbial and bacterial biomass (Supplementary material, Table S26) of evergreen and deciduous tree soils was about a third lower than in control forests (total microbial PLFA, age: $F_{1,16}$ = 115.18, $p < 0.01$; bacterial PLFA, age: $F_{1,16}$ = 76,74, $p < 0.01$). Within forests and old parks, total microbial PLFA biomass did not differ under evergreen and deciduous trees, but bacterial biomass was higher under deciduous ($F_{1,16}$ = 5.13, $p = 0.03$) than evergreen trees. Fungal biomass (Supplementary material, Table

S26) showed an interaction between land use type (control forest vs. urban park) and tree type ($F_{1,16} = 5.13$, $p = 0.03$): fungal biomass was similar among deciduous trees in forest and parks, whereas under evergreens it was substantially higher in forests than in parks.

The PLFA-based fungi/bacteria (F/B) ratios suggest that, regardless of park age and plant type, all soils were bacteria dominated (Fig. 1c). However, the relative proportion of fungi over bacteria was higher under deciduous trees than evergreen trees and lawns, regardless of park age (Table 1, Fig. 1c). None of the measured soil parameters (Supplementary material, Table S26) correlated with the F/B-ratio, and the F/B ratio among old parks and forests did not differ (Table 1).

The ratio between gram-/gram+ bacteria did not respond to park age or to vegetation type, and did not differ between control forests and old Lahti parks. However, the gram-/gram+ ratio correlated positively with soil pH (Table 1, Fig. 2d).

Relative abundances of PLFAs were used in NMDS analyses to investigate changes in the soil microbial communities in parks and control forest. Although microbial biomasses responded, we observed distinct changes in community composition only when the following datasets were analyzed; i) all plant types and park ages and ii) control forest and old deciduous and evergreen vegetation types in Lahti. When all plant types and park ages were included in the NMDSs, plant functional type had no effect on the soil microbial community composition in urban parks (Fig. 3, panel 1). However, park age influenced the composition of these communities ($R^2 = 0.06$, $p = 0.02$) (Fig. 3, panel 2). The soil microbial communities also differed ($R^2 = 0.11$, $p = 0.01$) between the two cities. Environmental variables – primarily % soil C ($R^2 = 0.14$, $p < 0.01$), % soil N ($R^2 = 0.12$, $p < 0.01$), the C/N-ratio ($R^2 = 0.08$, $p = 0.02$) and soil OM ($R^2 = 0.07$, $p = 0.02$) – correlated with the soil microbial community composition in the parks.

Comparing natural forest to deciduous and evergreen trees in old parks in Lahti showed that plant functional type clearly influenced ($R^2 = 0.14$, $p = 0.04$) the soil microbial communities (Fig. 3, panel 5). However, the soil

microbial community did not differ among old parks and control forests (Fig. 3, panel 6).

3.2. Nematodes

Bacterial and fungal feeding nematodes were more abundant under evergreen trees than under lawns (Table 1). However, while the abundance of bacterial feeders declined with park age, fungal feeders did not differ across park age classes. The abundance of bacterial feeders declined with park age and positively correlated with soil % soil C (Table 1). Fungal feeders negatively correlated with soil OM (Table 1). Plant feeders and omnivorous nematodes, although not responding to plant type or park age, positively correlated with soil OM and the C/N ratio (Table 1). When forests were compared with old parks in Lahti, bacterial feeding nematodes were more abundant ($F_{1,16} = 7.06$, $p = 0.01$) under evergreen trees than under deciduous trees in old parks and control forests, while the other groups did not differ (see supplementary material, Table S26).

Plant type affected the nematode channel ratio (NCR) ($F = 4.89$, $p = 0.01$). However, lawn and evergreen or deciduous and evergreen did not differ (Table 1, Fig. 4a), but rather, the differences can be attributed to those between deciduous trees and lawn. NCR negatively correlated with soil OM in the soil (Table 1). The NCR did not differ between old parks and control forest in Lahti. The maturity index (MI) showed a significant interaction between plant type and park age: when considering only deciduous trees, MI had the lowest values in young and old parks and highest values in intermediate parks (Table 1, Fig. 4b). MI also positively correlated with the soil C/N-ratio (Table 1). MI was higher ($F_{1,16} = 7.92$ $p = 0.01$) in old Lahti parks than forest controls (see supplementary material, Table S26).

We observed no relationships (Pearson correlations) between (i) the fungal marker and fungal feeding nematodes, (ii) bacterial biomass and bacterial feeding nematodes, and (iii) the PLFA fungal/bacterial-ratio and NCR.

The NMDSs revealed that nematode communities depended neither on plant functional type nor park age (Fig. 3, panels 7, 8). Park age did, however,

have an effect when deciduous and evergreen trees in old and intermediate parks were compared (Fig. 3, panel 10) (R^2 = 0.63, p < 0.01). Forests had distinct (R^2 = 0.48, p = 0.01) nematode communities compared to old Lahti parks (Fig. 3, panel 12). When all park samples were included in the analysis, % soil C was an important determinant of nematode community structure (R^2 = 0.07, p = 0.04). However, when only evergreen and deciduous trees in intermediate and old urban parks were investigated, only soil moisture correlated with nematode community structure (R^2 = 0.15, p = 0.04). Soil characteristics seemed to strongly control soil nematode communities (Fig. 1 panels 11, 12) when forests were compared to old parks, with only deciduous and evergreen trees included in the analysis (moisture: R^2 = 0.61, p < 0.01; pH: R^2 = 0.56, p < 0.01; OM: R^2 = 0.39, p = 0.01; % soil C: R^2 = 0.39, p = 0.01; % soil N: R^2 = 0.33, p = 0.02; C/N-ratio: R^2 = 0.57, p < 0.01). Nematode community compositions differed among the two cities when the comparison was made between all parks (R^2 = 0.13, p < 0.01) and when deciduous and evergreen trees in old and intermediate parks in Helsinki and Lahti were included in the analysis (R^2 = 0.19, p = 0.04).

3.3. Earthworms

GLMM results of the anecic earthworm *Lumbricus terrestris* showed that its biomass positively correlated with park age (Table 1), with the highest biomasses in intermediate and old parks (Supplementary material, Table S2). Plant type was not associated with *L. terrestris* biomass. *L. terrestris* correlated negatively with soil OM content (Table 1). The *L. terrestris* biomass did not differ between old parks and control forests in Lahti.

The epigeic and endogeic earthworm biomasses responded to plant type (Table 1), with highest abundances in lawns (Supplementary material, Table S2). Here, soil pH negatively correlated with the biomass of earthworms (Table 1). The biomass of these earthworm taxa did not differ between old parks and control forests, nor between evergreen and deciduous trees in the two habitat types.

4. Discussion

4.1. Effects of plant functional type on the soil biota

We hypothesized that different plant functional types – due to their documented divergent effects on soil characteristics and plant derived resources – will lead to changes in soil communities and the abundances of species and functional groups. Although overall communities were compositionally largely invariable, the biomasses and relative abundances of different microbial and nematode functional groups did respond to plant functional type.

Importantly, the PLFA-based F/B ratio was lowest in lawns, suggesting that bacteria dominated in lawn soils. Yet, to our surprise, soils under deciduous trees, and not evergreen trees, had the highest F/B and lowest NCR. NCR represents the relative abundance of bacterial feeders over fungal feeders, and thus mirrors the relative amount of bacteria over fungi in the soil (Yeates 2003). In general, the relative amount of fungi over bacteria tends to be higher under deciduous and especially under evergreen trees than lawn. Thus, our data contrast those reported (e.g. Wardle et al., 2004). The reasons for this remain unclear, and given our data, we can only speculate. For instance, a higher amount of fine roots (Giardina et al. 2005) and decaying roots support a higher saprophytic fungal biomass (Hobbie 2006) in soil associated with deciduous trees than evergreen, which could explain why soils associated with old lindens showed such a high F/B ratio. Another important factor potentially explaining the similar F/B ratio under lawns and evergreen trees may be the continuous disturbance present in urban parks; in particular, soil compaction can lower the F/B ratio (Hedlund et al., 2003).

Our third hypothesis focused on plant type mediated changes in higher trophic groups, such as nematodes and earthworm. GLMM analyses focusing on microbial and nematode feeding guilds revealed clear responses to plant type, although in some cases the response was seemingly mediated largely through age and soil properties of the parks. Total microbial biomass, as well as

bacterial and fungal biomasses separately were highest under deciduous trees in old parks, whereas no such effect was detectable in younger parks. Bacterial feeding nematodes and fungal feeding nematodes were more abundant under evergreen and deciduous trees than under lawn. Nematode communities often vary according to vegetation, as a direct result of plant-provided resources and indirectly through changes in the quality and quantity of the microbial fauna controlled by plant litter and exudate production. The lower abundance of nematodes in lawns may be due to diminished predation/consumption by earthworms (Dash 1980), which were less abundant under trees than lawns. Moreover, Yates (1981) reported complementary dynamics between earthworms and bacterial feeding nematodes, suggesting that nematodes and earthworms can compete for resources, with both using microbial biomass as food. In line with other studies, total microbial and bacterial biomasses in this study were also positively correlated with % soil C. Soil % C was also correlated with nematode abundance (both FF and BF) and community structure. Taken together, this is a clear indication that, just as in non-urban soils, the availability of carbon is a key determinant of microbial and secondary consumer biomass in urban soils, but that the effects appear only over time.

Contrary to previous findings (Lauber et al. 2009; Hui et al. 2017a), the soil microbial (PLFA) community did not correlate with pH. In contrast, the gram-/gram+ ratio did. Even though these two groups are not strict functional groups, they responded differently to soil pH; and in agreement with previous studies, gram- bacteria were relatively more abundant at high pH (Wang et al. 2016). Further, our results also corroborate Hui et al. (2017a), who showed that microbial communities (characterized with high throughput sequencing methods) in these urban parks were distinguished by plant functional types and correlated with % soil N and pH.

4.2. Effects of park age on the soil biota

Our fourth hypothesis stated that time since the establishment of a park (park age as well as tree age) will drive changes in the soil microbial and invertebrate communities. Our previous study, conducted in the same parks, but using high

throughput sequencing, showed that microbial communities differed compositionally between young parks and old / intermediate parks (Hui et al., 2017a). Consistent with those results, our NMDS analyses of microbial communities separated old and intermediate parks from young parks. This suggests that the soil microbial community reaches a stable structure between 10 and 50 years after park construction. Soil microbial community composition in old parks overlapped with that in control forests. However, microbial biomass was substantially higher in forests than in old parks. This is likely due to frequent disturbances in the latter system (Pickett and Cadenasso 2009). Malmivaara-Lämsä and Fritze (2003) reported that human soil trampling in an urban boreal forest did not affect microbial community composition. Accordingly, we also observed that microbial community composition of old parks approximates the non-disturbed forest sites, albeit microbial abundance is diminished in urban parks compared to forest sites. However, for logistical reasons, we sampled the urban parks and control sites (forest) at different times. Microbial biomass can change across seasons, with peaks in the spring and autumn (Diaz-Ravina et al. 1995). Nevertheless, the control sites also had higher soil OM and %C (Setälä et al. 2016) suggesting that the observed microbial biomass differences are unlikely mere artifacts attributable to different sampling times. In addition to biomass, microbial community composition can be seasonally dynamic (Moore-Kucera and Dick 2008), but despite such potential seasonal dynamics, the communities in control and urban park sites overlapped.".

Contrary to our expectations, the relative amount of fungi over bacteria did not increase as a function of park age. Similarly, NCR, describing the relative abundance of bacterial-feeding over fungal-feeding nematodes, remained mostly invariant across all park age classes. In successional systems, such as agricultural systems under restoration, the F/B ratio increases (Zeller 2001, Bailey 2002) as a result of changes in soil parameters (Zeller et al. 2001) and reduced disturbance (Hedlund et al. 2003). However, in urban parks the normal successional course is arrested and disturbance is continuous, possibly preventing increases in the relative abundance of fungi. Disturbance may also be the reason behind the invariant MI (Nematode Maturity Index) among the

parks of different ages. Interestingly, MI, which is regarded as a good indicator of ecosystem maturity, was higher in old parks compared with forest. In a study on the response of a riparian nematode community along a rural-urban transect, Pavao-Zuckerman et al. (2007) reported no differences in MI among sites, suggesting that the level of pollution was not high enough to influence MI. Also in our case, the levels of metal pollution were below ecologically relevant values (Setälä et al. 2017), but it is still unclear why MI values in forests are lower than in parks.

We were also unable to detect any shifts in the abundances of different nematode functional groups. Similarly to our speculations above, here too factors such as disturbance and litter removal may greatly contribute in altering resource availability to the soil biota, thus homogenizing these taxa across park ages. This could also explain the lack of response to plant traits and park age in plant-feeding nematodes, although plant root volumes likely increased substantially with time since park construction and establishment.

Nematode communities did respond to park age, but only in the rhizospheres of the two tree types; yet no changes in abundance with park age were observed. Nematodes can respond to changes in biotic and abiotic soil conditions (De Goede and Bongers 1994, Hanel 1995). Therefore, it is not surprising that we observed an age related shift in the nematode community only in association with trees, i.e. plant types which with time resulted in the clearest soil property changes in our study parks (see Setälä et al., 2016), and not in lawns.

The nematode community was also extremely responsive when we compared old parks and control forest, responding in parallel with the microbial communities as reported in Hui et al. (2017a). The nematode community structure was also correlated with changes in the soil characteristics, which in turn were modified by the different plant functional type and time. Community composition correlated with changes in abiotic soil parameters, such as pH, C, N and soil moisture. Again, this shows that nematodes are sensitive indicators (Neher 2001) of temporally mediated changes in soil characteristics. Contrary to previous findings, nematode densities were similar in rural forests and in urban parks. Pouyat et al. (1994)

suggested that a decline in nematode populations in urban soils might be linked to higher heavy metal concentrations in urban soils. On the other hand, Ohtonen (1992) found no link between metal pollution and nematode density. It is possible that pollution, and in particular metal loads, in urban soils in Helsinki and Lahti were not extreme enough to affect the soil biota.

Abundance of the anecic earthworm *L. terrestris* was lowest in young parks, increased with park age and reached a plateau in intermediate parks. The low abundance of earthworms in young parks can be attributed to two factors: i) lack of time for earthworms to colonize newly constructed habitats, and/or ii) the inhospitality of the newly built park soil as a habitat. As resources such as C and N increase in our park soils (see Setälä et al., 2016), so too does earthworm biomass. Similar trends in urban parks were observed by Smetak et al. (2007). This can have important implications since earthworms can ameliorate negative soil characteristics, compensating for and even reducing soil compaction with time. It is interesting to note that epigeic and endogeic earthworms did respond to pH variation, being more abundant at low pH than at high pH. Earthworms are quite sensitive to pH (Edwards and Bohlen, 1996). However, variation in pH among parks and plant types was rather narrow (Setälä et al., 2016) and among the earthworm species that we recorded, *Lumbricus rubellus* and *Aporrectodea caliginosa* were more abundant. Considering that pH is highly correlated with park age (Setälä et al., 2016), the significant relationship between earthworm abundance and pH could also be interpreted as the result of a relationship between park age and earthworm abundance.

Conclusions

Our data show that microbial biomass in natural forests is much higher than in urban parks, irrespective of their age. Thus, the urban greenspaces investigated unlikely approximate ecosystem properties or functions of the surrounding non-urban areas. Yet, our study provides strong evidence that deciduous trees support a greater microbial biomass with time since urban

green space establishment. Deciduous trees also had the highest relative amount of fungi over bacteria in urban parks, that evergreen trees would promote fungal rich soil microbial communities. Not only did microbial biomass increase with age in urban parks, but so too did the abundance of secondary consumers, such as earthworms. These data suggest that although urban parks do not approximate natural forests, likely as a consequence of the maintenance/disturbance regime they experience, the soil microbial and invertebrate communities respond to vegetation and edaphic shifts over time. Nevertheless, further studies are needed to assess the correlation and type of ecosystem services provided by these trophic groups.

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Figure and Table Captions

Fig. 1 The effects of plant functional type and park age on a) total microbial PLFAs, b) fungal PLFA (18:2ω6) and c) the PLFA fungal to bacterial ratio. Mean values ± SE are presented.

Fig. 2 Relation between a) the C/N ratio and predicted values of total microbial PLFAs, b) % soil C and predicted values of total microbial PLFAs, c) % soil N and predicted values of fungal PLFA (18:2ω6) and d) pH and predicted values of gram-/gram+ bacteria.

Fig. 3 NMDS plots for bacterial (1-6, on the left) and nematode (7-12, on the right) communities. Microbial communities were grouped by plant functional type (1, 3, 5) and age (2, 4, 6), and nematode communities were grouped by plant functional type (7, 9, 11) and age (8, 10, 12) under lawn, deciduous and evergreen trees in young, intermediate and old parks. Significant effects ($p < 0.05$) are indicated with a check mark in the upper panels and significant vectors are shown in the NMDS plots.

Fig. 4 The effects of plant functional type and park age on a) the nematode channel ratio (NCR), and b) the nematode maturity index (MI). Mean values ± SE are presented.

Table 1. GLMM results for PLFAs, nematodes and earthworms. For PLFAs the response variables included total microbial PLFA, bacterial PLFA, fungal PLFAs (18:2ω6, saprophytic fungi), fungal to bacterial ratio (fungi/bacteria) and gram + to gram – ratio (gram+/gram-). For nematodes the response variables included bacterial feeders, fungal feeders, plant feeders, predators and omnivorous, the maturity index (MI) and the nematode channel ratio (NCR). For earthworms the response variables were: *Lumbricus terrestris* and other earthworms. For each variable we reported the coefficient, standard error and p-value. Significant effects ($p < 0.05$) are highlighted in bold and significant interactions are indicated with an asterisk. Young evergreen trees are in the intercept.

Supplementary material, Table S2. Means (± 1 SD) of the following PLFAs: total microbial, bacterial, fungal PLFAs (18:2ω6, saprophytic fungi), fungal/bacterial ratio, gram/gram+ ratio, nematodes: bacterial feeders, fungal feeders, plant feeders, predators and omnivores, maturity index (MI), nematode channel ratio (NCR), earthworms: *Lumbricus terrestris* and other earthworms, for the three different park ages (young, intermediate and old) and plant functional type (evergreen, deciduous, lawn). Values for Lahti old parks and control forest (evergreen and deciduous trees) are also presented.

Table 1. GLMM results for PLFAs, nematodes and earthworms. For PLFAs the response variables included total microbial PLFA, bacterial PLFA, fungal PLFAs (8:2ω6, saprophytic fungi), fungal to bacterial ratio (fungi/bacteria) and gram + to gram – ratio (gram+/gram-). For nematodes the response variables included bacterial feeders, fungal feeders, plant feeders, predators and omnivorous, the maturity index (MI) and the nematode channel ratio (NCR). For earthworms the response variables were: *Lumbricus terrestris* and other earthworms. For each variable we reported the coefficient, standard error and p-value. Significant effects ($p < 0.05$) are highlighted in bold and significant interactions are indicated with an asterisk. Young evergreen trees are in the intercept.

	variable	intercept	lawn	deciduous	intermediate	old	age x functional group	pH	% soil C
PLFAs	Total microbial PLFAs	6.821	0.041	0.040	0.081	0.068			0.064
		0.220	0.092	0.092	0.103	0.100	*		0.023
		<0.001	0.447	0.437	0.791	0.675			**<0.001**
	Bacterial PLFAs	5.951	0.091	0.007	0.060	0.046			0.070
		0.228	0.094	0.094	0.105	0.102	*		0.023
		<0.001	0.336	0.942	0.565	0.655			**<0.001**
	Fungal PLFAs	3.414	0.129	-0.156	0.248	-0.320			-0.162
		0.290	0.266	0.261	0.272	0.274	*		0.085
		<0.001	0.627	0.549	0.362	0.243			0.056
	Fungi/bacteria	17.296	-4.994	8.044	-2.107	1.353			
		3.141	3.444	3.417	3.417	3.417			
		<0.001	0.147	**0.019**	0.537	0.692			
	Gram-/gram+	0.327	0.007	0.037	-0.064	-0.133		0.105	
		0.320	0.050	0.052	0.056	0.053		0.051	
		0.308	0.897	0.474	0.260	**0.018**		**0.040**	
Nematodes	Bacterial feeding	8.676	-2.038	-1.782	-2.121	-1.668			0.475
		2.279	0.847	0.832	0.860	0.852			0.218
		<0.001	**0.016**	0.321	**0.013**	0.050			**0.029**
	Fungal feeding	5.58	-1.322	-0.245	-0.35	-0.761			
		0.963	0.492	0.485	0.55	0.548			
		<0.001	**0.01**	0.612	0.524	0.165			
	Plant feeders	4.303	-1.030	-1.386	0.176	0.895			
		1.335	0.918	0.886	0.929	0.924			
		<0.001	0.262	0.118	0.849	0.333			
	Predators and omnivorous	-0.082	0.161	0.770	0.770	-0.109			
		1.049	0.438	0.438	0.462	0.463			
		0.937	0.712	0.104	0.095	0.813			
	MI	0.550	0.209	0.090	0.033	-0.074			
		0.119	0.059	0.060	0.064	0.064	*		
		<0.001	**<0.001**	0.134	0.600	0.246			

	NCR	0.418	0.008	-0.09	-0.084	-0.012	
		0.094	0.055	0.055	0.066	0.065	
		<0.001	0.121	0.084	0.212	0.848	
Earthworms	*Lumbricus terrestris*	1.920	-0.163	-0.242	1.452	1.097	-0.265
		0.725	0.320	0.319	0.406	0.404	0.123
		0.008	0.611	0.448	**<0.001**	**0.007**	**0.032**
	Other earthworms	3.657	0.794	0.433	0.160	0.368	-0.486
		1.355	0.190	0.196	0.226	0.217	0.200
		0.007	**< 0.001**	**0.027**	0.480	0.090	**0.015**

Table 2 Means (± 1 SD) of the following PLFAs: total microbial, bacterial, fungal PLFAs (18:2ω6, saprophytic fungi), fungal/bacterial ratio, gram/gram+ ratio, nematodes: bacterial feeders, fungal feeders, plant feeders, predators and omnivores, maturity index (MI), nematode channel ratio (NCR), earthworms: *Lumbricus terrestris* and other earthworms, for the three different park ages (young, intermediate and old) and plant functional type (evergreen, deciduous, lawn). Values for Lahti old parks and control forest (evergreen and deciduous trees) are also presented.

	Variable		Young	SD	Intermediate	SD	Old	SD	Old Lahti	SD	Control	SD
PLFA $\mu g\ g^{-1}$ soil dw	Total microbial PLFAs	Evergreen	380.09	132.83	526.96	208.32	417.16	93.44	318.82	64.09	842.19	319.05
		Deciduous	271.04	93.78	530.15	146.41	616.97	347.73	669.85	439.87	957.27	281.94
		Lawn	460.25	165.97	447.87	128.15	473.87	185.37				
	Bacterial PLFAs	Evergreen	172.85	66.11	223.54	88.39	181.19	50.83	160.42	46.90	799.71	306.51
		Deciduous	116.54	43.01	224.04	59.13	241.89	112.35	270.50	105.90	982.63	252.72
		Lawn	211.31	90.41	203.88	60.37	218.14	82.75				
	Fungal PLFAs	Evergreen	30.98	13.60	45.55	25.43	26.59	15.87	21.74	14.16	127.29	99.25
		Deciduous	24.65	9.00	48.12	28.67	111.03	125.14	141.06	156.35	127.97	100.76
		Lawn	34.26	12.77	23.88	11.22	26.16	16.65				
	Fungi/bacteria	Evergreen	20.33	12.39	21.17	11.73	15.96	11.25	14.05	8.43	18.39	15.22
		Deciduous	22.65	9.65	21.89	10.83	37.53	26.93	43.87	32.64	12.91	8.83
		Lawn	18.76	8.98	11.88	5.29	11.83	4.55				
	Gram+/gram-	Evergreen	2.98	0.45	2.77	0.31	2.49	1.02	3.07	1.02	3.14	0.83
		Deciduous	3.35	0.79	2.70	0.44	2.68	0.53	2.78	0.57	3.53	0.62
		Lawn	2.79	0.40	2.66	0.26	2.73	0.35				
Nematodes 10 g^{-1} soil	Bacterial feeders	Evergreen	139	113	100	85	93	47	64	33	74	31
		Deciduous	106	84	51	60	85	73	34	30	38	6
		Lawn	79	83	78	63	49	51				
	Fungal feeders	Evergreen	25	23	18	14	12	12	11	10	40	53
		Deciduous	24	25	20	30	13	9	12	7	13	8
		Lawn	11	11	10	14	7	6				
	Plant feeders	Evergreen	63	63	54	55	86	67	68	36	45	27
		Deciduous	37	32	43	53	48	33	47	36	16	24
		Lawn	129	283	69	55	136	285				
	Predators and omnivorous	Evergreen	9	8	12	9	7	11	4	4	19	15
		Deciduous	15	15	14	9	10	9	12	12	18	18
		Lawn	12	14	8	5	9	11				
	MI	Evergreen	2.17	0.11	2.30	0	2.35	0.23	2.42	0.03	2.15	0.20
		Deciduous	2.24	0.43	2.84	0.38	2.23	0.44	2.56	0.18	2.34	0.27
		Lawn	2.37	0.44	2.43	0.64	2.54	0.46				
	NCR	Evergreen	0.82	0.13	0.71	0.25	0.79	0.17	0.86	0.08	0.71	0.30
		Deciduous	0.77	0.19	0.85	0.12	0.88	0.09	0.70	0.17	0.76	0.12
		Lawn	0.88	0.12	0.88	0.10	0.87	0.10				
Earthworms $g\ m^{-2}$	*Lumbricus terrestris*	Evergreen	34	43	88	60	62	65	99	65	115	133
		Deciduous	15	32	63	49	66	67	102	77	97	100
		Lawn	27	30	54	67	43	37				
	Other earthworms	Evergreen	8	10	11	12	9	12	12	15	14	11
		Deciduous	9	13	19	32	19	13	25	13	50	48
		Lawn	16	16	36	44	35	40				

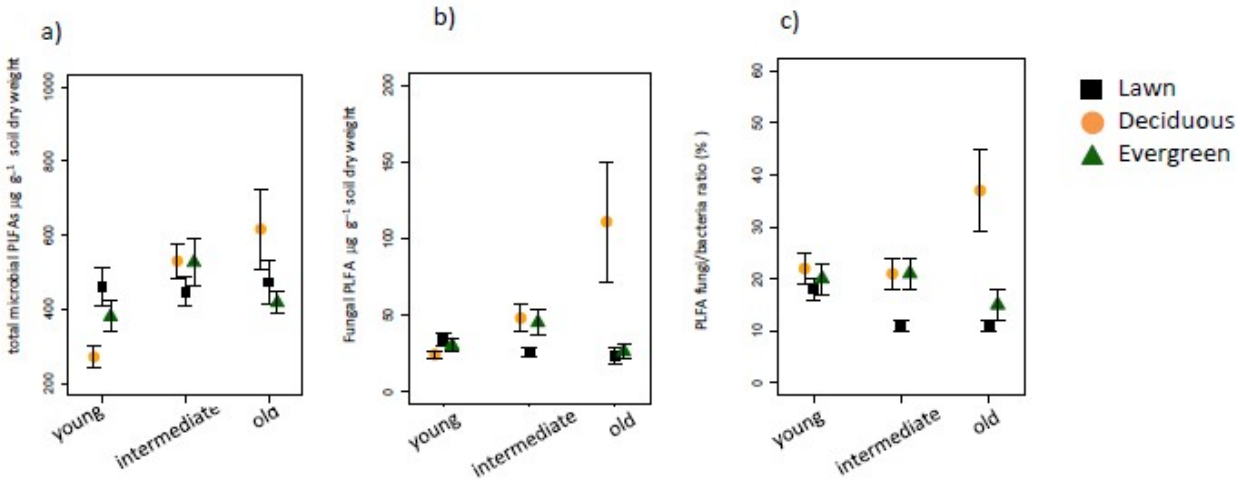
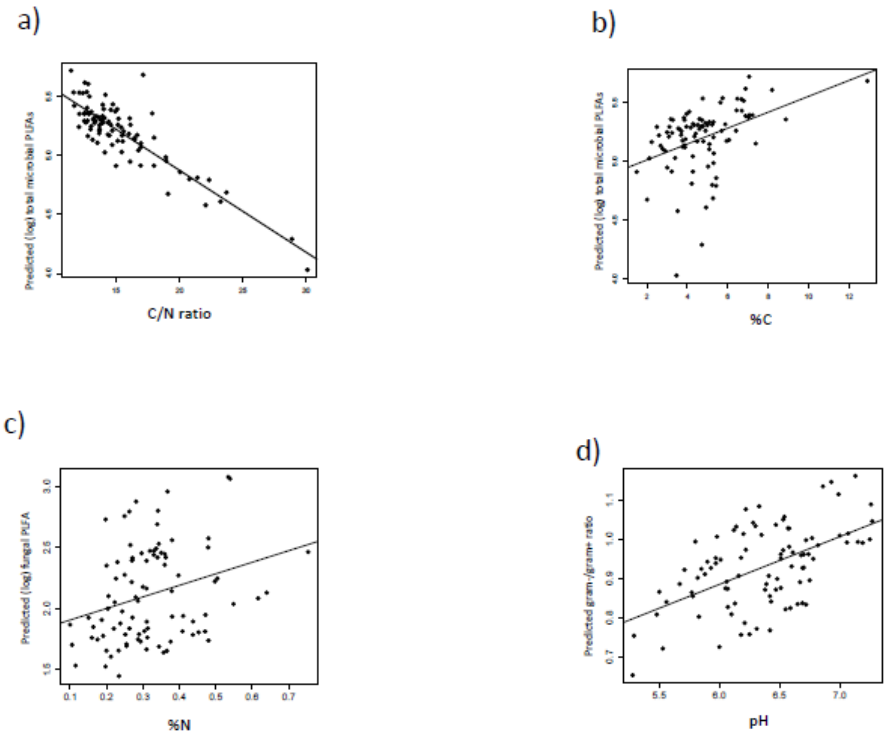

a)
b)
c)
total microbial PLFAs µg g⁻¹ soil dry weight
Fungal PLFA µg g⁻¹ soil dry weight
PLFA fungi/bacteria ratio (%)
young
intermediate
old
Lawn
Deciduous
Evergreen

Fig 1.



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1154Fig 2.

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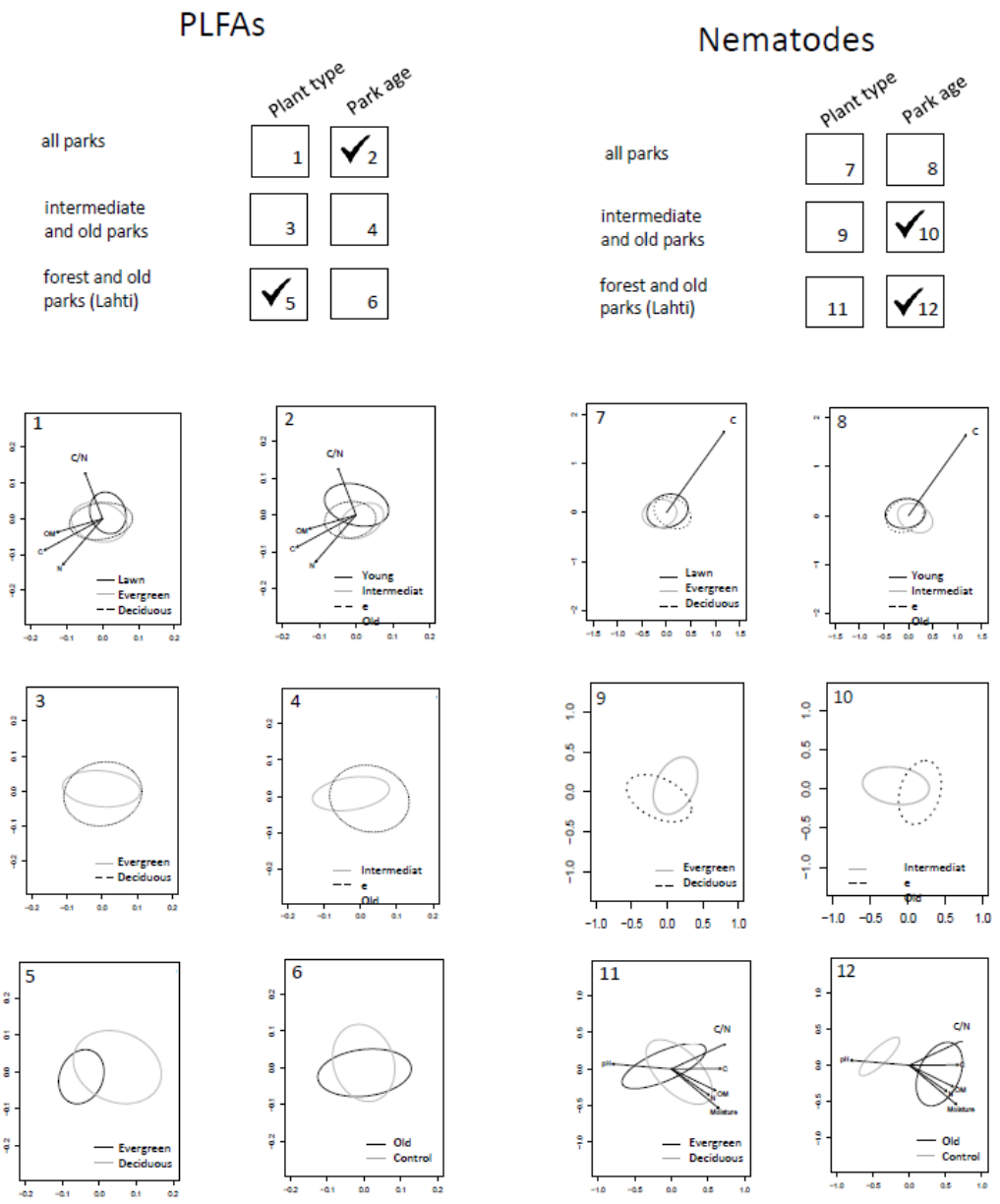

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1157Fig. 3

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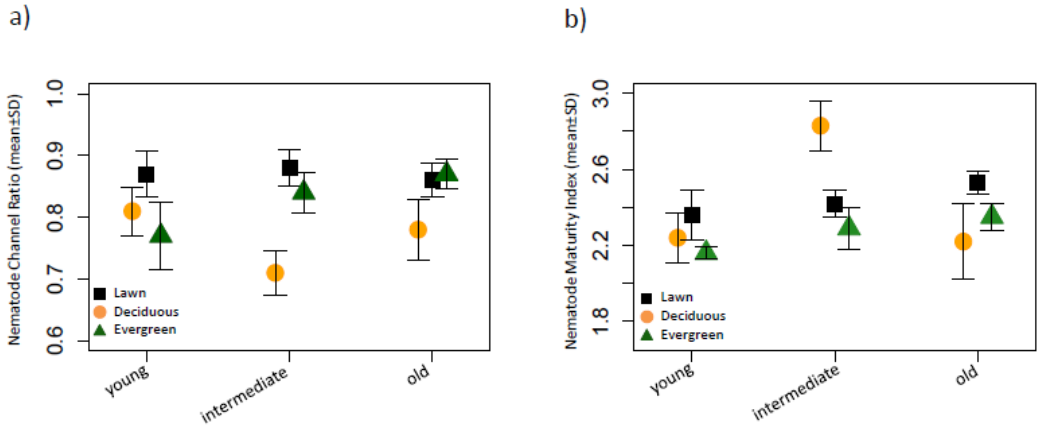

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1161Fig. 4

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